Methods for the differential diagnosis of rheumatoid arthritis

INAUGURAL-DISSERTATION

zur Erlangung der Doktorwürde
der Medizinischen Fakultät der Universität Bern

---

vorgelegt von Roland W. Sonntag
von Flushing, New York, U.S.A.

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Aus der medizinischen Klinik der Universität Bern
(Direktor: Prof. W. Hadorn)
Arbeit unter der Leitung von P.-D. Dr. M. Gukelberger

Aus dem Institut für Physiologische Chemie der Universität Bern
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(Direktor: Prof. C. Hallauer)
Arbeit unter Mithilfe von Herrn Dr. H. Löffler)

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PURPOSE

The differential diagnosis of various forms of Acute Polyarthritis, of Polyarthritis in quiesent stages with chronic arthrotic changes, and the task of approximating the degree of activity of various joint manifestations with any amount of certainty has long posed great difficulty.

Laboratory procedures now commonly in use, such as Erythrocyte Sedimentation Rates (E.S.R.), are often highly unspecific and are influenced by any number of insignificant factors. — Other procedures are more specific, although not completely so, and their reliability reaches only 70—85 %. Such tests are the Streptococcal Agglutination Titers which are positive in approximately 70 % of the Rheumatoid Arthritis cases (1), but also can be positive in Rheumatic Fever in relatively high percentages, and in cases of Scarlatina, acute Tonsillitis, and acute Nephritis (2).

It shall be the purpose of this paper to describe two methods, one specific for the diagnosis of Rheumatoid Arthritis, and one which shows clearly the activity of joint processes through the degree of tissue injury (3). Correlations, if any, between these two tests and with E.S. R. made simultaneously will be discussed and shown.

METHODS

The first of these tests is the Differential Agglutination Titer (D. A. T.) of Normal Sheep Erythrocytes (N. S. C.) and Sensitized Sheep Erythrocytes (S. S. C.). Various authors have reported on the high degrees of reliability and specificity of this test in the diagnosis of Rheumatoid Arthritis. Reliability in cases of active stages of Rheumatoid Arthritis has been reported as high as 97,9 % (4). High degrees of specificity, which are so important for differential diagnostic purposes have also been shown. (4 and 5)

Furthermore, it is of significance that Cortison Therapy does not affect the hemagglutinating factor which is bound on the Globulin Fractions of blood. (4) Therefore, the diagnosis can be made by this method even after hormonal therapy has been initiated. Although the D. A. T. values may be somewhat lower after the begin of therapy, they are still within positive ranges.

The procedure used for D. A. T. determinations has been that used by Rose et. al. (5) with the exception that horse anti-sheep cell serum was used instead of rabbit anti-sheep cell serum, and citrated sheep blood was used instead of defibrinated blood.

Preparations of N. S. C. and S. S. C. Suspensions :-

1- The fresh citrated sheep blood is washed at least three times in 0.85 % Saline. After the last centrifuging, a 1 % by volume suspension of the Erythrocytes is made in 0.85 % Saline. This is the N. S. C. Suspension used.

2- The hemolytic titer of the horse anti-sheep cell serum is determined. The dilution at which complete hemolysis of the Sheep Erythrocytes occurs is taken to contain one unit of hemolysin. The S. S. C. suspension is prepared by mixing a 2 % Sheep Erythrocyte suspension in Saline with an equal volume of Saline containing 2 units of Hemolysin.

Procedure :-

1- Serum of the patient is heated at 56° C. for 30 min. to destroy the complement.

2- From this heated serum, a duplicate series of doubeling dilutions is made in from 10 to 12 tubes with 0.5 ml. of Saline.

3- To each tube of one series 0.5 ml. of N.S.C. suspension is added. To the other series, 0.5 ml. of S. S. C. suspension is added to each tube.
The final dilution of patients serum in each series will then be 1 : 4 — 1 : 8 — 1 : 16 — 1 : 64 — etc.

4- The tubes are then placed in a water bath at 37° C. for 1 hour.

5- The tubes are then transfered to a refrigerator at 4° C. over night after which they can be read.

Reading :-

Each tube is flipped with the finger, and the degree of Agglutination is estimated with the naked eye.
4+ = a tightly packed clump of cells
1+ = a finely granular clumping of cells
The end point is each series is taken as the reciprocal of the dilution of serum in the last tube showing a 1+ Agglutination.

Example :-

Dilution —	4	8	16	32	64	128	256	512	1024
N. S. C.	3+	2+	1+	0	0	0			
S. S. C.	4+	3+	3+	2+	1+	1+	1+	0	0
D. A. T.				2	4	8	16		

In this case, the end point for the N. S. C. would be 16.
The end point for the S. S. C. would be 256.
The differential Agglutination Titer (D. A. T.) would be 256/16 or 16.

The second of these tests is the Determination of the Serum Diphenylamine Reaction. This is a colorimetric determination which is not specific for any one type of joint disease, but which gives a clear indication as to the activity of the disease as reflected through tissue injury.

The serum constituent which reacts with D. P. A. to give the purple color is as yet unknown. It seems to be constantly associated with

mucoprotein, and can be found not only in serum, but also in urine and synovial fluid. (3) Further work is in progress to determine the exact nature of this substance. However, it has been clearly shown that the D. P. A. rise in serum is intimately connected with the severity of inflamatory joint processes, and the number of joints involved. (3) Furthermore, it has also been shown that this D. P. A. rise can be depressed under salicylate, cortison, or splenin A. therapy. (3)

The procedure used for D. P. A. determinations is fundamentally the semi-micro procedure used by Coburn and Haninger (3) as described by Ayala, Moore and Hess. (6) this procedure is as follows :-

The reagents used are :-

(a) Reagent with D. P. A. — 1.0 gm. of D. P. A. (recrystallized from ethanol) dissolved in a mixture of 90 ml. glacial acetic acid and 10 ml. of concentrated sulfuric acid.

(b) Reagent without D. P. A. — the same proportions of acids as in (a), but without the D. P. A.

1- 5 ml. of a 5 % trichloracetic acid solution is added slowly, with gentle shaking, to a 0.2 ml. sample of serum. The tube is then stoppered with a rubber, or cork stopper which has been fitted with a 20 cm. long glass tube to act as a condenser, and placed in a boiling water bath for exactly 20 minutes.

2- After 20 minutes, the tube is immediately cooled by immersion in ice water. Following the cooling, the hydrolysate is filtered and 1.5 ml. of the clear filtrate is transfered to each of two fresh tubes respectively.

3- To the first of these tubes is added 3 ml. of reagent containing D. P. A. This will give the desired purple color. To the second of these tubes is added 3 ml. of reagent without D. P. A. This mixture will give a brownish color, the optical density of which must be subtracted from that of the purple color reaction in the first tube. This brownish color is caused by the action of sulfuric acid, and varies with each serum sample. It is, therefore, necessary to prepare this blank and determine its optical density separately for each individual serum sample in order to obtain a corrected optical density for the purple color D. P. A. reaction.

4- A second blank consisting of 1.5 ml. of 5 % trichloracetic acid and 3 ml. of Reagent with D. P. A., but no serum, is also prepared. This blank is used to establish the zero optical density setting on the spectrophotometer.

5- All these tubes, including the blanks, are immersed in a boiling water bath for exactly 30 minutes after which they are cooled in ice water and allowed to rise to room temperature. The color intensities which develop are stable at room temperature for several hours.

The spectrophotometer used in this study to determine optical densities was a Spekker of Hilger and Watts, London.

Orange filters 607, and containers giving a solution thickness of 1.0 cm. were used for all determinations. Readings were made on the logarithmic scale of the instrument.

In this study, an additional control was used as a check, and also in order to arrive at a final set of results which could easily be duplicated on various types of spectrophotometers. This consisted of 0.2 ml. of a 1 % glucose solution which is treated exactly as the 0.2 ml. serum sample. In other words, it was hydrolysed with 5 ml. of 5 % trichloracetic acid, and 1.5 ml. of the hydrolysate was treated with 3 ml. of reagent with D. P. A. A light blue color resulted, the optical density of which was also determined.

Gross changes in the optical densities of these sugar control solutions, which should remain fairly constant, would show either changes in the reagents used, or changes caused by heating too long, or too short a time. The practice of running such a sugar control simultaneously with each serum sample, or group of samples, not only provides a close check on the reliability of results, but also makes possible the determination of a Sugar Ratio (S. R.) for each serum sample. This S. R. which is the result of dividing the corrected optical density of the D. P. A. purple color by the optical density of the sugar control gives a numerical result of far greater significance than a mere instrumental reading, for it can be reproduced on any type instrument.

Serum samples used for D. A. T. and D. P. A. and S. R. determinations were obtained from the same venal puncture. The samples were refrigerated, but were never kept for longer than 24 hours. Erythrocyte Sedimentation Rates, made with blood from the same venal punctures, were made immediately.

RESULTS

A total of 45 serum samples were studied in this report. Of these 45 samples, 10 were normal and were used to determine the normal ranges of both the D. A. T. and the Serum D. P. A. Reactions. In contrast to Rose, (5) who found that active cases of Rheumatoid Arthritis never showed a D. A. T. of less than 16, it was found in this study that a D. A. T. of 8 or more is significant in the diagnosis of Rheumatoid Arthritis. Normal ranges for the Serum D. P. A. Reaction expressed in terms of Sugar Ratios (S. R.) were found to be from 2.72 to 3.52 in the 10 control sera. Sera showing S. R. values below 2.72 were not considered to be of special significance, as increased activity of a joint process should show increased rather than decreased S. R. values.

Table I

Table I shows the results of 10 normal sera in terms of E. S. R. in first and second hour readings, corrected optical density readings for the D. P. A. Reactions, Sugar Ratios and D. A. T.

Serum Sample	E. S. R.	D. P. A.	S. R.	D. A. T.
1	8/12	0.166	3.02	4
2	10/15	0.154	2.96	0
3	8/12	0.168	3.06	4
4	6/11	0.169	2.82	2
5	6/11	0.163	2.72	0
6	4/11	0.167	2.78	2
7	3/7	0.183	3.52	0
8	2/5	0.185	3.08	2
9	2/5	0.180	3.01	0
10	3/8	0.173	3.33	0

Next, the sera of 14 Rheumatoid Arthritis Cases were studied. Each case, with the exception of one, showed a D. A. T. of 8 or

higher. The one negative case, with a D. A. T. of 4, had no subjective symptoms at the time of testing. The only positive objective symptoms were a chronic swelling of the proximal phalangeal joints and high S. R. value. The diagnosis of Rheumatoid Arthritis was based on the history and previous reaction to specific Rheumatoid Arthritis Therapy. It was clearly shown that those patients with E. S. R. values of over 20 in the first hour all had a high S. R. value. See table II A and B.

Table II A

Rheumatoid Arthritis cases with E. S. R. over 20 in the first hour.

Patient	E. S. R.	D. P. A.	S. R.	D. A. T.
Miss E.	24/50	0.294	7.33	8
Mrs. T.	42/85	0.208	4.96	16
Miss S. L.	70/103	0.287	7.18	8
Mr. M. K.	95/130	0.280	7.00	8
Mrs. S. I.	59/99	0.298	7.47	32
Mr. B.	37/50	0.218	3.77	8
Mr. R. A.	110/140	0.280	4.92	32
Mrs. R.	63/91	0.258	3.86	32
Miss R.	36/70	0.217	3.62	8

Table II B

Rheumatoid Arthritis cases with E. S. R. below 20 in the first hour.

Patient	E. S. R.	D. P. A.	S. R.	D. A. T.
Miss. W.	12/17	0.161	2.52	8
Mrs. G. E.	15/21	0.224	3.50	16
Miss S. N.	12/30	0.179	3.89	4
Miss S.	17/	0.231	3.45	16
Mr. P.	15/	0.215	3.22	16

As a direct comparison to these Rheumatoid Arthritis cases, four cases of Acute Rheumatic Polyarthritis were studied. Each of these cases showed a very significantly raised S. R. value. Furthermore, all except one case had a D. A. T. value of 4 or less. See Table III. The one exception, with a D. A. T. of 16 was a 19 year old female with the following history :-

A year previous to testing, the patient suffered acute joint swelling in the small joints of the upper extremities. These mani-

festations followed an attack of tonsillitis and subsided somewhat on therapy. However, there was never a complete remission of the joint distrubances, and the E. S. R. remained constantly elevated. The proximal phalangeal joints were swollen, deformed, and showed definite restricted movement. Just prior to testing, the patient suffered acute exacerbations of her joint manifestations with high fever. Treatment with salicylates proved successful in combating the temperature. Five days after testing, cardiac involvement showing extensive myocardial damage were found in the electrocardiogram recordings.

It is quite evident that last exacerbation was an Acute Rheumatic Process, and it has been shown by Nanna Svartz (4) that in such cases, the D. A. T. may be temporarily positive. However, in view of the long history (over 12 months) of joint manifestations without cardiac involvement, it is very likely that this patient had been suffering from a Rheumatoid Arthritis to which an Acute Rheumatic Arthritis with myo and endocarditis had been superimposed.

Table III

Cases of Acute Rheumatic Polyarthritis.

Patient	E. S. R.	D. P. A.	S. R.	D. A. T.
Mr. R.	45/77	0.293	5.14	2
F. G.	125/135	0.364	5.87	2
Mr. S.	55/66	0.250	5.21	2
Miss L. K.	140/160	0.317	6.22	16

It is also of interest to mention in this group a case of Streptococcal Sepsis with Ostoemyelitis. This patient showed multiple migrating joint manifestations with a typical septic fever curve. Blood cultures, X-Ray, and finally surgury confirmed the diagnosis. At the time of testing, the following values were recorded:- E. S. R. — 63/104, D. P. A. — 0.345, S. R. — 6.39 and D. A. T. — 2.

Various forms of Arthrosis were also studied. Because of the similar results obtained with cases Morbus Bechterew, they shall also be included in this group. In none of these cases was a D. A. T. of over 4 observed. S. R. values were inconstant, but no exceedingly high values were noted. See table IV.

Table IV

Disease	Patient	E. S. R.	D. P. A.	S. R.	D. A. T.
Coxarthrosis	Mrs. K.	16/20	0.190	2.97	0
	Mr. H. F.	2/3	0.150	2.82	2
	Mrs. S. R.	20/55	0.242	4.83	2
Spondylarthrosis	Mrs. B. N.	9/19	0.180	2.91	4
	Mr. P.	6/13	0.241	3.88	2
Osteochondrosis	Mrs. W.	12/19	0.187	3.12	0
	Mr. P.	20/30	0.198	3.30	2
Morbus Bechterew	Mr. M. W.	14/19	0.183	2.77	2
	Mr. H. W.	5/14	0.239	4.51	0
	Mrs. L. I.	20/45	0.204	3.28	4
	Mr. B.	4/12	0.235	3.78	2

In this group of cases, it is especially clearly shown that the E. S. R., S. R. and D. A. T. values have no relationship what so ever to one another.

In order to enlarge the spectrum of diseases studied, the following cases were also tested. See table V:-

(a) Two cases of Periarthritis Humero-Scapularis. Both patients showed a D. A. T. of 2, and S. R. values bordering on the upper limits of normal.

(b) One case of Pseudopolyarthritis caused by neurological disturbances in the Plexus Brachialis.
The resulting neuro-vascular changes of the upper extremities caused an objective picture closely resembling that of a Rheumatoid Arthritis. This patient also had a D. A. T. of 2, and a S. R. value slightly above normal.

(c) Two cases of Acute Gouty Arthritis. Both these patients exhibited high S. R. values. One had a D. A. T. of 2, and the other had a D. A. T. of 16. Although the diagnosis of Gouty Arthritis was perfectly clear at the time of testing, the patient with the high D. A. T. had a history of previous joint manifestations which remained unclear. The possibility of a simultaneous Rheumatoid Arthritis may be augmented by the history, sex (female), and extremely high E. S. R. (69 in the first hour) at the onset of the manifestations.
In any case, the number of Gouty Arthritic patients observed in this study does not permit a definite conclusion to be drawn as

to the value of D. A. T. determinations in their diagnosis. However, it should be pointed out here that as yet, authors employing the D. A. T. method of diagnosis have not reported consistently high D. A. T. values in cases of Gouty Arthritis.

Table V

Disease	Patient	E. S. R.	D. P. A.	S. R.	D. A. T.
Periarthritis Humero-Scapularis	Mrs. S. A.	5/	0.194	3.34	2
Periarthritis Humero-Scapularis	Mrs. S. B.	7/	0.208	3.59	2
Pseudo-Polyarthritis	Mr. I. S.	3/13	0.198	3.88	2
Acute Gouty Arthritis	Mr. K.	104/	0.274	4.42	2
	Miss H.	5/	0.202	3.92	16

DISCUSSION AND CORRELATIONS

From the results obtained in this study, it has been shown that the D. A. T. determinations provide a reliable method for diagnosis of Rheumatoid Arthritis. This method was shown to be especially valuable in differentiating this disease from other types of chronic and acute joint diseases. It's value is further enhanced by the fact that several patients showing positive D. A. T. had been under cortison therapy for periods of two weeks and more prior to testing. Although the tests were not 100% reliable and consistant, it was clearly seen that their reliability and consistancy was far superior to those of the E. S. R. reaction. See tables II A and B. It may also be pointed out that biological tests in general rarely show greater diagnostic reliability.

In the case of Rheumatoid Arthritis patients, a certain correlation between the D. A. T. and E. S. R. reactions was observed. (See tables II A and B.) All the patients showed high E.S. R. results, and all, with the exception of one, (discussed above) showed significantly raised D. A. T., It is, however, to be noted that the degree of E. S. R. elevation was in no manner correlated to the degree of D. A. T. elevation. Furthermore, it was seen in the studies of joint diseases other than Rheumatoid Arthritis, that the D. A. T. values were hardly ever found to exceed those considered normal, and that they in no way corresponded to the E. S. R. values. See tables III and IV.

The Serum D. P. A. levels expressed in this study in term of Sugar Ratios (S. R.) were not found to be consistantly high in cases of Rheumatoid Arthritis. However, all active cases of this disease did show raised S. R. values as seen in table II A. Although inactive cases showed variations in the S. R. determinations, the variations were not extreme when compared with the variations seen in control patients, (see tables I and II B) and exceedingly high S. R. values were not encountered in this group. The cases of Acute Rheumatic

Polyarthritis studied showed invariably high S. R. values. Other groups of joint diseases studies showed considerable variations in the S. R. values. These variations were seen not only between the various disease groups, but also within the individual groups themselves.

Correlations between S.R. values and E.S.R. values may be concluded inasmuch as an elevated E. S. R. generally has an elevated S. R. value. However, once again the degrees of elevation of these two tests could not be correlated further. Correlation between S. R. values and D. A. T. values were not found either except possibly in cases of Active Rheumatoid Arthritis where the elevation of one was accompanied by an elevation of the other. Here again, the degrees of elevation of the two tests could not be successfully correlated.

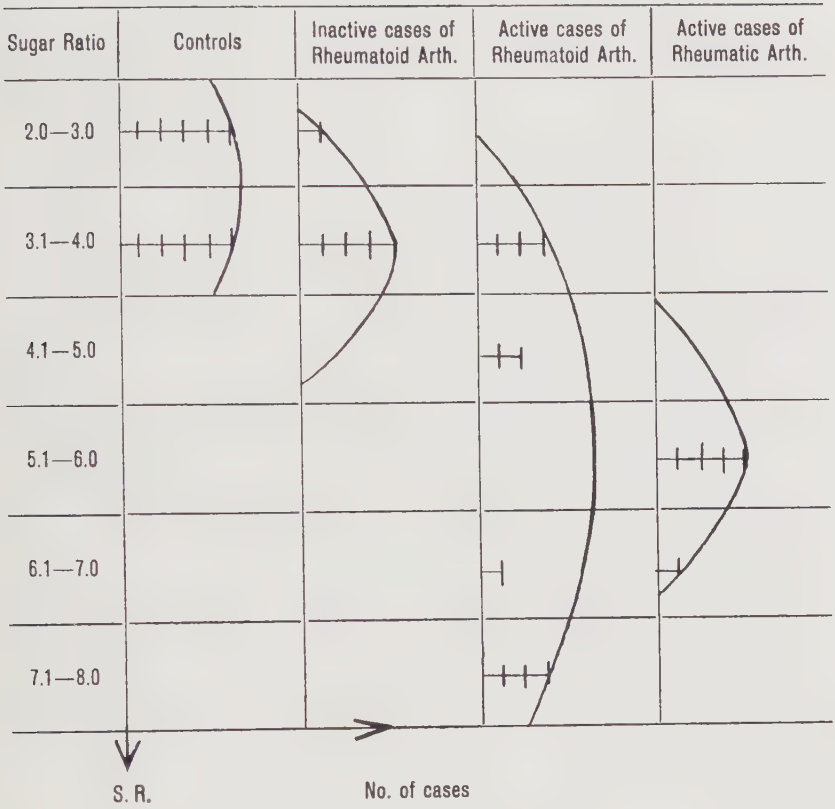
CONCLUSION

It is clearly shown by the results of this study that the D. A. T. determination provides a definite aid in the diagnosis of Rheumatoid Arthritis. Although 100 % reliability was not observed, the specificity of this test was found to be far superior and more reliable than those methods commonly used at present for diagnosis. Not only was this test reliable when applied after longer periods of therapy, but also when applied during periods of inactivity of the disease.

The determination of S. R. values as derived from Serum D. P. A. Reaction values proved definitely of value in estimating the activity of joint processes. It was clearly shown in this study that this reaction is in no way specific for any one type of joint disease. The method may be used to determine the activity of any type of joint manifestation regardless of its etiology. The effect of cortison on this reaction has been shown by Coburn (3). This effect may be used to evaluate therapeutic measures and progress. It has been observed in this study that although the S. R. values may decrease under cortison therapy, these values will remain significantly high as long as the disease continues to produce objective and subjective symptoms.

In figure I, S. R. values are plotted against the number of Cases exhibiting these values. The cases have been grouped according to diseases for comparison. A marked tendency toward increase in the number cases with elevated S. R. values is seen in the disease groups of an active nature. This figure is not for the purpose of demonstrating definite values, but to show a definite trend in S. R. rise in joint diseases with active processes.

Figure I


Sugar Ratio
Controls
Inactive cases of Rheumatoid Arth.
Active cases of Rheumatoid Arth.
Active cases of Rheumatic Arth.
2.0—3.0
3.1—4.0
4.1—5.0
5.1—6.0
6.1—7.0
7.1—8.0
S. R.
No. of cases

ZUSAMMENFASSUNG

Zweck der Untersuchungen:

Die vorliegende Arbeit befasst sich mit der Auswertung von zwei neuen Serumreaktionen auf dem Gebiete der Rheumatologie.
Es liegen folgende 2 Fragestellungen zu Grunde:

1. Gelingt die serologische Unterscheidung der primär chronischen Polyarthritis von andern Gelenkerkrankungen mit Hilfe des Agglutinationsunterschiedes von normalen Schaferythrocyten einerseits und sensibilisierten Schaferythrocyten andrerseits ?
2. Eignet sich die Diphenylamin-Reaktion des Serums zur Bestimmung der Intensität eines Gelenkprozesses ?

Während die erste Methode von N. Svartz, Stockholm, als mehr oder weniger spezifische Methode zur Erkennung der primär chronischen Polyarthritis in die Rheumatologie eingeführt worden ist, handelt es sich bei der zweiten um eine ganz neue Serumreaktion, die bis anhin nur für den Nachweis von kollagenem Bindegewebe gebraucht worden ist.

Methodik:

Die Agglutination der normalen und sensibilisierten Schaferythocyten mit dem Patientenserum erfolgte nach der Methode von Rose, Ragan, Pearce und Lipman, die Bestimmung der Diphenylaminreaktion im Serum nach den Verfahren von Ayale, Moore und Hess.

Ergebnisse:

Normale Seren zeigen einen Differentialagglutinationstiter von 0-4, wenig aktive Fälle von primär chronischem Gelenkrheumatismus einen solchen von 8-16, aktive Formen einen solchen von 8-32, während Fälle von akutem Gelenkrheumatismus mit niedrigen Werten von 2 einhergehen. Ebenfalls niedrige Werte von 0-2

zeigten sich bei den Fällen mit primär degenerativem Rheumatismus. Von den 45 Werten machten nur 2 eine Ausnahme, wobei in einem Fall eine Gicht, im andern eine akute arthritische Phase bei einem primär chronischen Gelenkrheumatismus zur Diskussion standen.

Die Diphenylamin-Reaktion des Serums — eine Glucosaminreaktion in zuckerwerten ausgedrückt — zeigte kein krankheitsspezifisches Verhalten. Bei allen rheumatischen Krankheitsgruppen kamen niedrige und hohe Werte vor. Die Reaktion kann viel mehr als Mass der Intensität der Erkrankung angesehen, wobei man an das Ausmass der Untergangs von kollagenem Bindegewebe denken kann.

Schlussfolgerungen:

1. Die Bestimmung des Agglutinationsunterschiedes (Differentialagglutinationstiter) von normalen und sensibilisierten Schaferythrocythen mit Patientenserum eignet sich in hohem Masse zur Differentialdiagnose der primär chronischen Polyarthritis. Von unseren 45 Untersuchungen wichen nur 2 von den erwarteten Resultaten ab, wobei es sich allerdings nicht sicher um reine Fälle von primär chronischer Polyarthritis gehandelt hat.

2. Die Diphenylaminreaktion ist nicht krankheitsspezifisch; sie gibt ein Mass für die Intensität des arthritischen Prozesses.

BIBLIOGRAPHY

1- CECIL, R. L. and DE GARA, P. F., the Agglutination Reaction for Heamolytic Streptococci in Rheumatoid Arthritis. — It's Significance in Diagnosis and Treatment, Am. J. M. Sc., 211 : 472, 1946.

2- THULIN, K. E. — Sweden, Streptococcal — o — Agglutination, Summary of Communications VIII International Congress of Rheumatic Diseases.

3- COBURN, A. F. and HANINGER, J., The Serum Diphenylamine (D. P. A.) Reaction in Experimental Arthritis, Journ. of Exp. Med., Vol. 99, No. 1, pp 1—9, Jan. 1, 1954.

4- SVARTZ, N. — Sweden, Agglutination with Sensitized Sheep Erythrocytes, Summary of Communications VIII International Congress of Rheumatic Diseases.

5- ROSE, H. N., RAGAN, C., PEARCE, E., and LIPMAN, M. O., Procedure of Differential Agglutination Titer. Determination and Specificity Thereof., Proc. Soc. Exp. Biol. and Med., 68 : 1, 1948.

6- AYALE, W., MOORE, L. V., and HESS, E. L., Semimicro Method of D.P.A. Determination, J. Clin. Inv. 30, 781, 1951.